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DR. CHARLES B. JORDAN

1878 - 1941

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JUNE
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IN MEMORIAM

Charles B. Jordan

WITH the passing of Dean Charles B. Jordan on April 22, 1941, Pharmacy lost one of its outstanding leaders.

Dr. Jordan had a long and interesting career in both the field of education and research. He was graduated from Michigan State Normal College in 1904 and served as Superintendent of Schools in his home town for four years. He graduated from the University of Michigan in 1910 and from their Graduate School in 1912. Dean Jordan began his career as a pharmaceutical educator with his appointment as Professor of Pharmaceutical Chemistry at Purdue University in 1910 and in 1924 he was appointed Dean of the School of Pharmacy.

Dr. Jordan's contributions have been many. Much of the progress in raising the standards of pharmaceutical education are due to his efforts. He was an active worker in the various professional and scientific organizations of pharmacy and was three times elected as a member of the Revision Committee of the United States Pharmacopœia which work he conducted with tireless and whole-hearted effort. His publications in the field of pharmaceutical research are legion and many are those who have had the privilege of working under his able leadership.

Many honors and honorary degrees were given him in recognition of his extensive professional contributions during his very active career.

This Journal is indeed itself honored to dedicate this issue to the memory of one whose place in pharmacy cannot readily be filled.

EDITORIAL

NUTRITION AND NATIONAL HEALTH

These pertinent paragraphs are prompted by pharmacy's problems. They are intended for the best interest of pharmacy and public health.

OUR government has initiated a program which is intended to increase the health of the nation by providing better nutrition, particularly to those who by reason of low income can least afford it. Such steps as the expansion of the food stamp plan to include certain low income groups, making available additional protective foods and enriching bread with thiamin, etc., have been suggested. Although this step is tied in with our national defense effort, it appears that this is largely a sugar-coating for the benefit of some "rugged individualists" who look upon any governmental activity to protect its people as an unwarranted transgression upon their inalienable rights. It is true that some of the "New Deal" activities have been of questionable merit but here is a program that no fair-minded person can criticize, at least as far as its aims are concerned.

Many of us who are well fed and, in addition, in possession of the necessary knowledge and finances to guarantee proper nutrition of ourselves and our families are apt to overlook the real problem of one who has both low income and a large family for which to provide. This poor fellow must see his children, who are just as dear to him as our own are to us, fail to grow properly, suffer dental caries and endure all of the other vicissitudes which we know are caused by poor nutrition. How can such an individual without government help remedy this situation?

Not only is the problem an economic one but one calling for effective propaganda to acquaint all people in this great country of ours with the vital importance of good nutrition. The advertising of vitamin products has overdone the job in certain population groups but, unfortunately, the persons who listen and who can afford to buy these products probably need them the least. There are indeed groups wherein even a joint program of making better foods available plus propaganda will be ineffective. It is a well known fact that certain vitamin deficiencies disturb the intellection of the individual. He no

longer has judgment nor can he make necessary decisions. Such cases demand supervision by public health workers and treatment and choice of diet cannot be, and should not be, voluntary. Equality of opportunity is an empty phrase when applied to a pellagrin who in this vicious circle is almost helpless. If our democracy is to survive we must solve some of these problems within which are just as dangerous as enemies from without.

Now, let us examine the effect of this new program on the sale of vitamin preparations throughout the country. Some of the many unethical vitamin products will probably be hurt. We can well afford to lose them since they are not only an economic waste but they discredit many of the efforts and products of reliable manufacturers who are trying in a sincere manner to benefit public health. The consumption of ethical vitamin products will probably not decrease greatly, in fact the attendant publicity given to better nutrition may even increase their use.

There will always be two groups who will need to take vitamin products; those whose diet is restricted by whim or physical necessity and those whose vitamin requirements are abnormally high through metabolic derangement. In addition, we will always have a host of persons who as a matter of being certain that their supply is adequate will feel constrained to regularly use such products.

One problem that surely needs clarification is at what point does a product cease to become a food and fall within the category of drugs? Since high potency vitamin preparations may be demonstrated to have great potential metabolic and even pharmacologic power, their sale in grocery stores and elsewhere would appear to be extremely hazardous. The pharmacist who is trained to appreciate this fact and who is in a position to advise and guide his clientele as to their proper use is the logical one to supply these drugs. Pharmacy is glad to cooperate in furthering the highly commendable governmental program of raising the nutritional standards of the country through better diet, but it does take the stand that high potency vitamin products are drugs and not foods.

Pharmacists, generally, should make every effort to assist those responsible for the execution of this broad program for better nutrition. In serving as a source of *reliable* information, not tinctured with thought of personal gain, the pharmacist can go far in bringing to his doorstep that public esteem which comes to everyone who is willing to render real public service.

L. F. Tice.

SURFACE TENSION REDUCENTS IN BACTERICIDAL SOLUTIONS: THEIR IN VITRO AND IN VIVO EFFICIENCIES*

The following two papers present some very interesting data on the effect of surface tension depressants on the efficiencies of bactericidal agents. Such variables as surface tension, hydrogen-ion concentration and their interrelation are discussed and the results of skin penetration tests and toxicity studies are given.

By Louis Gershenfeld † and Bernard Witlin †

THE killing of a bacterial cell by a bactericide in solution is, in addition to other factors, dependent most frequently upon the diffusion of the bactericidal substance through the cell wall (10). This process is complicated by the fact that one substance (the protoplasm) is separated from another (the bactericide) by a semi-permeable membrane (the cell wall) (27, 31, 32). It is evident therefore, that the bactericidal agent to be active must be in solution and that any factor that would tend to increase its rate of diffusion through the cell wall would, in all probability increase its bactericidal efficiency (6). It has been reported (15, 16, 17) that when some substances, which have the property of being surface tension depressants, are added to a bactericidal solution, both the rapidity of penetration and the actual bactericidal efficiency of the bactericide are markedly increased, even though the depressant itself which was added has no toxic action on the test organism. This phenomenon is said to be due to an increase in the rate of diffusion of the bactercide into the bacterial cell (7), the velocity of the diffusion being increased by the reduction of the surface tension (29, 30).

It appears logical to assume that lowering the surface tension, which increases the rate of diffusion, must play some part in increasing bacterial cell destruction by bactericides. Experimental evidence demonstrating this fact has been presented by Berczeller (3, 4); Hansen (22); Billard (5, 6); Billard and Dieulafe (7); Liese and Mendel (33); Larson (25); Larson, Cantwell and Hartzell (26); Leonard and Frobisher (29, 30); Frobisher (15, 16, 17);

* The publication of this research was delayed for more than a year to await the completion of another investigation on a surface-tension reducent appearing in this issue.

† Department of Bacteriology, Philadelphia College of Pharmacy and Science.

Rideal (38); Bechold and Reiner (1); Stanley, Coleman, Green, Sacks and Adams (43); Stanley and Adams (42); Eisenberg (14); Cowles (12, 13); and Scales and Kemp (39).

Scheurlen (40), Beckman (2) and Reichel (37) found that the addition of sodium chloride to a solution of phenol markedly increased its bactericidal activity. Wolf (49) reported that the addition of sodium ricinoleate as the surface tension depressant increased the bactericidal efficiency of substances against only some species of bacteria. On the other hand, Hansen (22) reported inhibitory effects of disinfectant solutions upon the addition of saponin and soaps; Hampil (21) found that sodium soaps decreased the bactericidal action of phenol, meta-cresol and secondary butyl-alcohol; and Tilley, McDonald and Schaeffer (44) noticed the decrease in the bactericidal efficiency of phenols upon the addition of glycerin.

Recently, a large variety of surface tension depressants have been made available for use as wetting agents, penetrants, detergents, emulsifying and spreading agents (46). These products are employed by many industries among which are those concerned with textiles, leather, insecticides, paper, ceramics, pharmaceuticals, metals, paints, cosmetics, oils and gases. This general class of chemical compounds first found application in the textile field. Their use commercially dates back to the interest in the development of sulfonated oil substitutes and soaps with greater hydrolytic and detergent properties.

Purpose:

In view of the modern conception of the workings of surface tension depressants (9, 24, 48), there was reason to suspect that their addition to antiseptic solutions would increase the bactericidal efficiency of such solutions and also exhibit interesting and useful properties of surface activity in combination (20). It was with this problem in mind that this research was undertaken.

In Vitro Technique

Surface Tension Depressants Employed:

Fifty-four marketable (so-called) surface tension depressants which are employed commercially as wetting agents were secured (see "A" under Addenda). Preliminary tests were conducted for the selection of the most representative depressants depending upon their compatibility with the antiseptic itself, stability in aqueous or

alcoholic solutions, absence of excessive soapiness, absence of objectionable odor, color and reaction and one low in cost.

Of the fifty-four depressants, the following were selected as the most representative:

1) Aerosol OT
2) Aerosol MA
3) Aerosol AY
4) Aerosol OS
5) Alkanol B
6) Alkanol SA
7) Calgon
8) Calagon (Unrefined)
9) Daintex
10) Dreft
11) Drene
12) Duponol ME
13) Duponol WA
14) Glycerol-mono-oleate
15) Invadine C
16) Invadine NC
17) Invadine N
18) Nacconol LA
19) Nacconol NRSF
20) Penetrex
21) Pine Oil 1
22) Pine Oil 9
23) Santomerse D
24) Santomerse 3
25) Sulphatate
26) Sulfonated Castor Oil 50%
27) Sulfonated Castor Oil 75%
28) Tergitol 4
29) Tergitol 7
30) Tergitol 08
31) Terposol 8
32) Triethanolamine
33) Triton B
34) Ultrawet
35) Ultrawet 40
36) Ultravon W
37) Viriform A
38) Yarmour Pine Oil 302
39) 2-amino-2-ethyl-1, 3 propanediol

(A):

One per cent. aqueous solutions of these thirty-nine depressants were made and Berkefeld filtered under aseptic conditions. One-half cubic centimeter of the filtrates was transplanted into flasks containing 100 cc. of sterile dextrose-veal infusion boullion and incubated at 37 degrees C. for one week. Portions were subsequently kept at room temperature for eighteen months. All solutions not showing growth were regarded as sterile. These sterile Berkefeld-filtered solutions were stored in sterile rubber-stoppered liter pyrex bottles and from the latter the solution of the depressant was added under aseptic conditions to the different antiseptics (as will be noted later). Attention also is directed to the fact that the age of the respecive depressant solutions at the time of the actual testing was not considered during this investigation.

FINDINGS: *The results obtained in these tests revealed that the one per cent. solutions prepared were sterile.*

(B):

Kolmer's bacteriostatic test was employed to determine their efficiencies (23).

FINDINGS: *The following depressants were revealed, by the foregoing test, to be bacteriostatic:*

1) Tergitol 4
2) Dreft
3) Drene
4) Duponol WA
5) Invadine B
6) Invadine C
7) Invadine N
8) Ultravon W

(C):

The following, having been used as surface tension depressants by various workers (2, 17, 19, 21, 22, 25, 35, 37, 40, 44) also were examined at the same time, employing 1 per cent. aqueous solutions (see Addenda "A" for source):

1) Sodium ricinoleate
2) Sodium undecylenate
3) Sodium laurate
4) Sodium palmitate
5) Sodium chloride
6) Glycerin

FINDINGS: *All of the six agents tested, listed under (C), were found to be non-bacteriostatic in 1 per cent. aqueous solutions.*

List of Bactericidal Agents Employed

I) PHENOLIC DERIVATIVES:

1) Phenol (Merck and Company). An accurately assayed 5% aqueous solution.
2) *o*-Cresol (Barrett and Company). 1 per cent. aqueous solution of, made water-miscible with sulfonated castor oil (18).
3) *m*-Cresol (Barrett and Company). 1 per cent. aqueous solution of, made water-miscible with sulfonated castor oil (18).

4) *p*-Cresol (Barrett and Company). 1 per cent. aqueous solution of, made water-miscible with sulfonated castor oil (18).
5) Cresol, U. S. P. XI (Merck and Company). 1 per cent. aqueous solution of, made water-miscible with sulfonated castor oil (18).
6) Beta-naphthol (Baker Chemical Company). 1:1000 aqueous solution.
7) Resorcinol (Merck and Company). 5 per cent. aqueous solution.
8) Hexylresorcinol Solution 1:1000 (S. T. 37) (Sharp and Dohme, Inc.).
9) Thymol (Schering Corp.). 1 per cent. alcohol-acetone solution.*
10) Chlorthymol (Merck and Company). 1 per cent. alcohol-acetone solution* diluted 1:100 with distilled water (final concentration 1:10,000).†
11) Menthol (Merck and Company). 1 per cent. alcohol-acetone solution*.
12) *o*-Phenylphenol (Dow Chemical Company). 1 per cent. alcohol-acetone solution*.
13) Salicylic Acid (Merck and Company). 1 per cent. alcohol-acetone solution*.
14) Carvacrol (Eastman Kodak Company). 1 per cent. alcohol-acetone solution*.
15) Chlor Symmetrical Xylenol (Barrett and Company). 1 per cent. alcohol-acetone solution*.
16) Para Chlor Meta Cresol (Barrett and Company). 1 per cent. alcohol-acetone solution*.

II) Mercurial Derivatives:

17) Mercuric Bichloride (Merck and Company). An accurately assayed 1:1000 aqueous solution.
18) Mercury Oxycyanide (Merck and Company). 1:1000 aqueous solution.

* Alcohol-acetone solutions were made according to the following formula: Ethyl Alcohol (95%), 55 parts; Acetone (USP), 9.5 parts; Bactericidal Substance, 1 part; Distilled Water sufficient to make 100 parts.

† The preparation was diluted for the purpose of obtaining the end-point of *in-vitro* bactericidal efficiency. In *in-vivo* procedures they were employed in their original and undiluted concentrations.

19) Merthiolate, 1:1000 Tincture (Eli Lilly & Company).
20) Merthiolate, 1:1000 Solution (Eli Lilly & Company).
21) Merphenyl Borate, 1:500 Tincture (Hamilton Laboratories).
22) Merphenyl Nitrate, 1:1500 Aqueous (Hamilton Laboratories).
23) Metaphen, 1:200 Tincture (Abbott Laboratories).
24) Metaphen, 1:500 Solution (Abbott Laboratories).
25) Mercurochrome (Hynson, Westcott and Dunning). 2 per cent. aqueous solution prepared.
26) Mercresin, 1:1000 Tincture (Upjohn Company).

III) Halogen Derivatives:

27) Tincture of Iodine (U. S. P. XI). Assayed 5 per cent. Iodine, diluted 1:20 with distilled water before use†.
28) Zonite (Zonite Products Company). Diluted 1:380 with distilled water immediately before use†.

Bactericidal Efficiency Tests

(D):

Employing a standard strain of Staphylococcus aureus No. 209 (45) bactericidal efficiency tests were performed as follows (36):

Nine dilutions of the bactericidal agents were made in sterile distilled water. Starting with the medicament (S), the nine dilutions prepared were each one-half the strength of the preceding dilution.

In sterile cotton stoppered test tubes, 0.5 cc. of the Staphylococcus aureus of standard resistance (45) was added to 5 cc. of the increasing dilutions of each antiseptic (36). After remaining in a water bath at 37 degrees C. for five minutes, one 4 mm. loopful was transferred to 10 cc. of F. D. A. broth (45). All tubes were incubated at 37 degrees C for forty-eight hours. The effective killing dilution was the highest dilution from which transfers failed to exhibit growth. In the case of the Mercurials, the contents of all tubes failing to show growth were transferred to flasks containing 100 cc. of F. D. A. broth and incubated for another forty-eight hours at 37 degrees C. to test for bacteriostatic action.

Findings: *See Table No. 1.*

† The preparation was diluted for the purpose of obtaining the end-point of *in-vitro* bactericidal efficiency. In *in-vivo* procedures they were employed in their original and undiluted concentrations.

Table 1

BACTERICIDAL EFFICIENCIES

S. aureus for Five Minutes at 37° C.

Antiseptic Solutions	Dilutions									
	S	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512
Phenol (1:20 aqueous)	−	−	−	+	+	+	+	+	+	+
o-Cresol (1:100 aqueous)	−	+	+	+	+	+	+	+	+	+
m-Cresol (1:100 aqueous)	−	+	+	+	+	+	+	+	+	+
p-Cresol (1:100 aqueous)	−	+	+	+	+	+	+	+	+	+
Cresol (USP——1:100 aqueous)	−	+	+	+	+	+	+	+	+	+
Beta-naphthol (1:100 aqueous)	+	+	+	+	+	+	+	+	+	+
Resorcinol (1:20 aqueous)	−	+	+	+	+	+	+	+	+	+
Hexylresorcinol (1:1000) S. T. 37	−	−	−	+	+	+	+	+	+	+
Thymol (1:100 AA*)	−	−	−	−	−	+	+	+	+	+
Chlorthymol (1:100 AA*) (1:100)	−	−	−	+	+	+	+	+	+	+
Menthol (1:100 AA*)	−	−	−	−	−	+	+	+	+	+
o-Phenylphenol (1:100 AA*)	−	−	−	−	−	+	+	+	+	+
Salicylic Acid (1:100 AA*)	−	−	−	+	+	+	+	+	+	+
Carvacrol (1:100 AA*)	−	−	−	−	−	+	+	+	+	+
Chlor Symmetrical Xylenol (1:100 AA*)	−	−	−	−	−	−	+	+	+	+
Para Chlor Meta Cresol (1:100 AA*)	−	−	−	−	+	+	+	+	+	+
Mercuric Chloride (1:1000 aqueous)	−	−	−	+	+	+	+	+	+	+
Mercury Oxycyanide (1:1000 aqueous) ×	+	+	+	+	+	+	+	+	+	+
Merthiolate (1:1000 Tincture)	−	−	−	+	+	+	+	+	+	+
Merthiolate (1:1000 Solution)	−	−	−	+	+	+	+	+	+	+
Merphenyl Borate (1:500 Tincture)	−	−	−	−	−	+	+	+	+	+
Merphenyl Nitrate (1:1500 aqueous)	−	−	−	−	+	+	+	+	+	+
Metaphen (1:200 Tincture)	−	−	−	−	+	+	+	+	+	+
Metaphen (1:500 Solution)	−	−	−	−	−	−	+	+	+	+
Mercurochrome (1:50 aqueous)	−	−	+	+	+	+	+	+	+	+
Mercresin (1:1000 Tincture)	−	−	−	−	−	+	+	+	+	+
Tincture of Iodine USP XI (assayed 5% I) (1:20)	−	−	−	−	−	−	−	−	−	+
Zonite (1:380)	−	−	−	−	−	−	−	+	+	+

− : no growth after 48 hours incubation at 37° C.
\+ : growth after 48 hours incubation at 37° C.
AA* : Alcohol-acetone solution.
S : Medicament in strength as indicated in first column.
× : These findings though checked and rechecked were always as noted herein. An assay was not made. The container of mercury oxycyanide bore the manufacturers' batch number and recently upon inquiry we were advised that the composition of this batch which was apparently old was entirely different from that of mercury oxycyanide as marketed at present. In fact the mercury oxycyanide from our first source may have been decomposed, which accounts for these findings for the oxycyanide throughout this research.

(E):

Surface tension measurements were conducted at the same time on all solutions employed by use of the DuNouy Tensiometer. The surface tension measurements were taken of all the antiseptic solutions used (S) and of the last dilution thereof, killing S. aureus (45) at 37 degrees C. in five minutes by itself and after the culture was added. The surface tension of the distilled water, F. D. A. broth and the standard 24-hour culture of Staphylococcus aureus (45) also were determined.

FINDINGS: *See Table No. 2.*

(F):

The hydrogen-ion concentration (pH) determinations were performed by means of a Beckman Electric Potentiometer on all solutions (S) and on the last killing dilutions of these antiseptics.

FINDINGS: *See Table No. 3.*

(G):

The highest dilution killing one-half cubic centimeter of Staphylococcus aureus in five minutes having been noted, similar tests were performed using the same bactericidal agents in combination with the surface tension reducing agents. The thirty-nine depressants were added separately to each of the original dilutions of all products labelled (S) in the concentration of 1:100, 1:500 and 1:1000 parts.

The products (S) containing the previously mentioned concentrations of depressants were diluted with sterile distilled water to obtain the dilution employed in the bactericidal efficiency tests.

FINDINGS: *The bactericidal solutions thus treated did not show any increase in their bactericidal efficiencies as compared with the original solutions to which depressants were not added.*

(H):

To further determine whether or not there would be an increase in the efficiency of these bactericides upon the addition of surface tension depressants they were retested, the original bactericides containing a concentration of 1:1000 parts of the respective depres-

Table 2

SURFACE TENSION MEASUREMENTS

Antiseptic Solution	Surface Tension Dynes/sq. cm. at 25° C.	Last Dilution Killing S. aureus in 5 Minutes at 37° C.	Surface Tension of Last Dilution Killing S. aureus in 5 Minutes at 37° C. in H_2O	Surface Tension of Last Dilution Killing S. aureus in 5 Minutes at 37° C. plus S. aureus
Phenol (1:20 aqueous)	40.00	1:4	41.50	42.00
o-Cresol (1:100 aqueous)	37.50	S	37.50	37.50
m-Cresol (1:100 aqueous)	37.00	S	37.00	37.00
p-Cresol (1:100 aqueous)	37.00	S	37.00	37.00
Cresol (USP——1:100 aqueous)	37.00	S	37.00	37.00
Beta-naphthol (1:1000 aqueous)	51.75	....	....	....
Resorcinol (1:20 aqueous)	60.25	S	60.25	60.25
Hexylresorcinol (1:1000) S. T. 37	34.25	1:4	37.50	37.50
Thymol (1:100 AA*)	30.80	1:16	38.50	38.50
Chlorthymol (1:100 AA*)	27.50	1:4	49.00	49.00
Menthol (1:100 AA*)	27.00	1:16	44.00	44.00
o-Phenylphenol (1:100 AA*)	32.00	1:16	52.00	52.00
Salicylic Acid (1:100 AA*)	31.00	1:2	38.50	38.50
Carvacrol (1:100 AA*)	33.00	1:16	45.00	45.00
Chlor Symmetrical Xylenol (1:100 AA*)	32.00	1:32	51.00	51.50
Para Chlor Meta Cresol (1:100 AA*)	32.50	1:8	43.00	43.00
Mercuric Chloride (1:1000 aqueous)	71.50	1:4	70.00	68.50
Mercury Oxycyanide (1:1000 aqueous)	70.50	....	....	....
Merthiolate (1:1000 Tincture)	32.00	1:4	51.00	51.50
Merthiolate (1:1000 Solution)	71.25	1:4	71.00	60.00
Merphenyl Borate (1:500 Tincture)	32.50	1:16	68.00	58.00
Merphenyl Nitrate (1:1500 aqueous)	71.00	1:8	68.00	56.00
Metaphen 1:200 Tincture)	30.50	1:8	54.50	55.00
Metaphen (1:500 Solution)	67.00	1:32	72.00	66.00
Mercurochrome (1:50 aqueous)	71.50	1:2	71.50	65.00
Mercresin (1:1000 Tincture)	31.00	1:16	39.00	39.00
Tincture of Iodine USP XI (assayed 5% I (1:20)	50.50	1:256	59.50	53.50
Zonite (1:380)	70.50	1:64	70.50	54.50
Controls:				
Distilled Water	71.25 (Theoretically 71.97 dynes per sq. cm. at 25° C.)			
F. D. A. Broth	50.50			
Standard S. aureus Culture in Broth	50.50			

S : Medicament in strengths as indicated in first column.
.... : less than Stock Solution, no further determinations made.
AA* : Alcohol-acetone solution.

Table 3

HYDROGEN-ION CONCENTRATION DETERMINATIONS

Antiseptic Solution	pH of Solution	Last Killing Dilution for S. aureus in 5 Minutes	pH of Last Killing Dilution for S. aureus at 37° C. in 5 Minutes
Phenol (1:20 aqueous)	5.70	1:4	6.33
o-Cresol (1:100 aqueous)	8.00	S	8.00
m-Cresol (1:100 aqueous)	7.83	S	7.83
p-Cresol (1:100 aqueous)	7.80	S	7.80
Cresol (USP——1:100 aqueous)	7.80	S	7.80
Beta-naphthol	6.66	....	....
Resorcinol	6.00	S	6.00
Hexylresorcinol (1:1000) S. T. 37	3.35	1:4	3.80
Thymol (1:100 AA*)	6.00	1:16	6.83
Chlorthymol (1:100 AA*)	5.70	1:4	6.26
Menthol (1:100 AA*)	6.80	1:16	6.62
o-Phenylphenol (1:100 AA*)	6.80	1:16	6.98
Salicylic Acid (1:100 AA*)	2.80	1:2	3.20
Carvacrol (1:100 AA*)	5.42	1:16	5.95
Chlor Symmetrical Xylenol (1:100 AA*)	5.66	1:32	6.50
Para Chlor Meta Cresol (1:100 AA*)	5.88	1:8	5.98
Mercuric Chloride (1:1000 aqueous)	5.33	1:4	5.02
Mercury Oxycyanide (1:1000 aqueous)	6.20	....	....
Merthiolate (1:1000 Tincture)	9.00	1:4	8.82
Merthiolate (1:1000 Solution)	9.92	1:4	9.62
Merphenyl Borate (1:500 Tincture)	5.85	1:16	5.58
Merphenyl Nitrate (1:1500 aqueous)	4.66	1:8	5.20
Metaphen (1:200 Tincture)	10.90	1:8	9.42
Metaphen (1:500 Solution)	11.00	1:32	10.25
Mercurochrome (1:50 aqueous)	8.65	1:2	8.40
Mercresin (1:1000 Tincture)	6.82	1:16	5.40
Tincture of Iodine USP XI (assayed 5% I) (1:20)	5.00	1:256	6.40
Zonite (1:380)	9.90	1:64	9.50
Alcohol-acetone "blank" solution	6.40	....	....
70% Ethyl Alcohol	6.75	....	....
Distilled Water	6.65 (Theoretically pH 7.0)		....
F. D. A. Broth	6.80	....	....

S : Medicament in strengths as indicated in first column.
AA* : Alcohol-acetone solution.
.... : Less than Stock Solution, no further determinations made.

sants. All dilutions subsequently employed were made with sterile 1:1000 aqueous solutions of the depressants under examination, instead of sterile distilled water, so that the respective depressants were present in all dilutions in a 1:1000 concentration.

This was repeated with concentrations of 1:100 and 1:500, keeping the respective amounts of the thirty-nine depressants in all dilutions constant throughout the tests.

FINDINGS: *The results in all instances did not reveal different findings in the bactericidal efficiencies of the antiseptics to which the depressants had been added.*

In Vivo Technique

(A):

Though there was no increase in the *in vitro* efficiencies of bactericidal solutions upon the addition of non-bacteriostatic surface tension reducents there was the possibility that *in vivo* tests would reveal different findings. The following experiments were performed employing the same bactericidal agents with a representative group of surface tension depressants (see Addenda "B").

Using normal young 1000 gram rabbits (because of their rapid healing tendencies) the abdomens were clipped and shaved 24 hours in advance and the animals replaced in their individual cages so as to obtain as large a yield as possible of the normal flora of the skin.

The animals were anaesthetized with ether and a 50 by 50 mm. square was swabbed for 30 seconds with 2.5 cc. of the antiseptic solution saturated on sterile-cotton-wrapped wooden applicators. The solutions were allowed to remain on the skin for 3 minutes and a puncture wound was made with a 5/16-inch diameter sterile skin trephine. The skin, held between sterile Forbe's cleft palate forceps, was immersed and rinsed in a tube containing 20 cc. of 1 per cent. dextrose-veal infusion broth, to remove any excess antiseptic, then placed into another 20 cc. tube of the broth which was also incubated at 37 degrees C. for seven days and examined for sterility.

One-tenth cubic centimeter of a standard Staphylococcus aureus culture (45) was applied to the wound by means of a sterile pipette. Numerous reports are available concerning the inability to obtain a virulent strain of S. aureus to retain uniform virulence throughout a set of experiments. The standard F. D. A. Staphylococcus aureus

(45) was therefore employed. This was allowed to dry and five minutes later 2.5 cc. of the antiseptic was swabbed over the same area. The animal was replaced in its cage without any protective covering on the wound and 2.5 cc. of the antiseptic swabbed over the wound 12, 24, 36 and 48 hours after removal of the tissue. The condition of the wound was examined at the time of swabbing and daily thereafter.

Upon complete healing of the wound, the animal was again anaesthetized, the cicatrice removed, fixed in Zenker's solution, paraffin embedded, sectioned at 7 microns on a rotary microtome and stained by Meyer's modification of Ehrlich's Tri-acid Stain for granulation (28). Controls were run without bactericidal agents, employing a 70 per cent. solution of ethyl alcohol and an alcohol-acetone solution.

This study was made on each of the bactericidal solutions by themselves and on the latter containing 0.5 per cent. of each of the depressants (see Addenda "B"). An attempt was made to determine the following:

1) The efficiency of antiseptics for skin disinfection and the production of skin sterility by chemical means.

2) The effect of surface tension depressants upon the bactericidal solutions in skin sterilization.

3) The effect of surface tension depressants in combination with bactericidal solutions on wound healing.

FINDINGS: *In not one instance was there complete sterility. Disinfection of the skin was a variable factor, varying in the same disinfectant and from experiment to experiment. All wounds healed within twelve to fifteen days regardless of the antiseptic or depressant solution used. Controls upon which no antiseptics were applied did not become infected and healed in the same period of time.*

Histological studies of scar-tissue presented normal observations: Hypertrophy of the granular layer was absent; the basic stratum was normal; there was no degeneration of the Malpighian layer; and perfect normality in all strata.

(B):

To further test the bactericidal efficiency *in vivo*, of these solutions, skin-sterility tests were carried out as follows: The animals

were anaesthetized with ether and a 50 by 50 mm. square was swabbed for 30 seconds with 2.5 cc. of the antiseptic solution saturated on sterile cotton-wrapped wooden applicators. The solutions were allowed to remain on the skin and at time intervals of 5, 10 and 15 minutes from the time of application 5/16-inch portions of skin were removed by use of a sterile skin trephine and a pair of Forbe's cleft-palate forceps. The skin was held between the sterile forceps, immersed and rinsed in a tube containing 20 cc. of 1 per cent. dextrose-veal infusion broth, to remove any excess antiseptic, then placed into another 20 cc. tube of the broth which was also incubated at 37 degrees C. for seven days and examined for sterility.

FINDINGS: *All tubes containing the skin showed growth microscopically, mainly of the spore-bearing type. No portion of skin exposed to the antiseptic solutions during the time period revealed complete sterility. Disinfection of the skin was a variable factor, varying in the same disinfectant and from experiment to experiment.*

Toxicity of Depressants Employed (In Vivo Tests)

(C):

Normal 2000 gram rabbits were acclimated for a period of two weeks and the following tests performed upon the normal animals:

1) Total Erythrocyte Count (RBC) (41)
2) Total Leucocyte Count (WBC) (41)
3) Differential (Wright stained smears) (41)
4) Haemoglobin Determinations (Newcomber's method employing a Klett Photoelectric Colorimeter for comparison) (41).

The animals were injected intravenously slowly with 20 cc. of Berkefeld filtrates of 1 per cent. solutions of the surface tension depressants (see Addenda "B") made in sterile physiological saline.**

Twenty-four hours later the complete routine blood examinations were again performed.

**100 milligrams per kilogram of body weight were used.

FINDINGS: *There was no observable change in the blood picture. Controls of 1 per cent. soap solutions caused haemolysis upon intravenous injection due to the saponin therein contained, resulting in death of the animals thus injected.*

(D):

To further check the toxicity, 50 cc. quantities of the preceding solutions (see Addenda "B") were injected intraperitoneally into 2000 gram rabbits.***

FINDINGS: *All animals thus injected, lived. No observable changes in weight nor change in blood pictures were discernible.*

(E):

2000 gram rabbits were force fed by placing the depressants through a 3/8-inch hole, drilled through a 5/8-inch wooden dowel, into the back part of the mouth; deglutition was forced by closing the rabbits mouth and covering the nostrils with the palm of the hand. 500 milligrams of each of the depressants (see Addenda "B") were fed separately to each individual animal.

FINDINGS: *There was no loss of weight, normal appetite and activity persisted. Blood pictures remained normal and constant.*

Skin Penetration Tests

(F):

Upon the assumption that the penetration of chemical substances is increased by the lowering of their surface tension, a series of experiments were performed with mercuric chloride**** (1:1000 aqueous) and arsenic trioxide***** (1:500 aqueous) alone, and in combination with the selected depressants (see Addenda "B").

Previous experimentation (11, 47) reported that chemical substances did not enter the body through the normal skin, but that:

***250 milligrams per kilogram of body weight were used.

* Each application was equivalent to approximately:

****Mercuric Bichloride : 0.5 mgm./kgm. of rabbit.

*****Arsenic Trioxide : 1.0 mgm./kgm. of rabbit.

1) Mercurials were vaporized and inhaled.

2) In inunctions there was a chemical combination with the lipoids in the pores and sweat glands.

3) In highly alkaline substances there might be a breaking of the skin and entry made therefrom.

The abdomens of 2500 gram rabbits were clipped and the animals placed in individual metabolism cages. At two hour intervals, five cubic centimeters of the above concentrations were swabbed over the complete abdominal area for thirty seconds. Samples of urine and faeces were collected and tested for heavy metals by the Reinsch Test (34). This procedure was conducted for a period of seven days on each animal. As controls, 1.0 cc. of the different solutions was injected subcutaneously and samples of urine and faeces were collected and examined.

FINDINGS: *The controls were Reinsch positive. All other urine and faeces collected were Reinsch negative.*

Summary

1) A comparative *in vitro* study of the bactericidal activity, bacteriostatic action, hydrogen-ion concentration and the surface tension of twenty-eight antiseptic solutions as used in practice was made.

2) Sterility tests, bactericidal efficiency and Kolmer bacteriostatic tests were conducted on thirty-nine surface tension reducing agents commonly employed as wetting agents.

3) A comparative *in vitro* study was made of the bactericidal activity of these twenty-eight antiseptic solutions (listed in 1) to which 0.1 per cent., 0.5 per cent. and 1.0 per cent. of the individual thirty-nine surface tension reducing agents (listed in 2) had been added. The surface tension depressants were sterilized by Berkefeld filtration, stored in sterile rubber-stoppered liter pyrex bottles and from the latter the solution of the depressant was added under aseptic conditions to the different antiseptics. The age of the different depressant solutions varied and was not considered during this investigation.

It is of special interest to note here that pH determinations were not made of the mixtures nor were the bactericidal studies conducted under different hydrogen-ion concentrations.

4) A comparative study of the skin disinfecting and sterilizing effects of twenty-eight antiseptic solutions, with and without the addition separately of twelve surface tension reducing agents and various controls was made on rabbit skin.

5) A comparative *in vivo* study of the effect of twenty-eight antiseptic solutions upon wound healing was made with and without the addition separately of twelve surface tension reducing agents and various control agents.

6) A study of the penetrating powers through the skin of rabbits was conducted with solutions of mercuric chloride and arsenic trioxide to which twelve surface tension reducing agents and various controls were added separately.

7) Toxicity tests of twelve surface tension reducing agents and various control agents were conducted on rabbits.

Conclusions

From the foregoing experiments, the following findings are noted:

1) The addition of thirty-nine available marketable surface tension reducing chemicals, commonly employed as wetting agents, in concentrations of 0.1 per cent., 0.5 per cent. and 1.0 per cent. respectively to the antiseptic dilutions of sixteen phenolic, ten mercurial and two halogen compounds, (and without making adjustments to specific hydrogen-ion concentrations) did not increase their bactericidal or bacteriostatic efficiencies.

Attention is directed to the fact that in this investigation the adjustment to specific hydrogen-ion concentrations was not considered, and little stress was made concerning the age of the solutions of the various surface tension depressants. As an outgrowth of this,

another investigation reported by Gershenfeld and Perlstein (50) in this issue of this Journal, indicates that at least with a specific surface tension reducent, these two factors appear to be of great importance in the final results obtained.

2) Regardless of whether antiseptic solutions were or were not applied to experimentally produced puncture wounds in normal young rabbits, the wounds healed by primary repair within 12 to 15 days. The use of twenty-eight antiseptic solutions containing separately 0.1 per cent., 0.5 per cent. and 1.0 per cent. of the twelve surface tension reducing agents and various controls (unadjusted to specific hydrogen-ion-concentrations) did not appear to affect the healing rate of experimentally produced puncture wounds in rabbits.

3) The addition of twelve wetting agents to aqueous solutions of mercuric chloride and arsenic trioxide did not increase the penetration of the inorganic compounds when applied to the unbroken skin of rabbits.

4) Sterilization of uniform pieces of rabbit skin exposed to the antiseptic solutions, with and without the addition of twelve surface tension reducing agents and various control agents was not accomplished. Disinfection of the skin was a variable factor, varying in the same disinfectant and from experiment to experiment.

5) The toxicity of the twelve wetting agents employed appeared to be negligible, since rabbits were capable of withstanding large doses intravenously, intraperitoneally and *per Os,* without displaying any change in weight, activity or in the blood picture over a period of three months.

Acknowledgment

This research was supported by a grant from Sharp & Dohme, Inc. We wish to extend our thanks to them and to all who supplied information and experimental quantities of the chemicals used.

ADDENDA "A".

No.	Product	Type	Manufacturer
(1)	Aerosol OT-100%	Sulfonated ester of dicarboxylic acid	American Cyanamid and Chemical Corporation
(2)	Aerosol OT-10%	"	"
(3)	Aerosol MA	"	"
(4)	Aerosol AY	"	"
(5)	Aerosol OS	Alkyl aryl sulfonate	"
(6)	Ultrawet	Aromatic monosodium sulfonate	Atlantic Refining Co.
(7)	Ultrawet 40	"	"
(8)	Alkanol B	Sodium alkyl naphthalene sulfonate	E. I. duPont de Nemours and Company
(9)	Alkanol S. A.	"	"
(10)	Duponol ME	Long-chain fatty alcohol sulfate	"
(11)	Duponol WA	"	"
(12)	Merpol B	Long-chain alcohol sulfate	"
(13)	Tergitol 7	Higher secondary alcohol	Carbide and Carbon Chemical Co.
(14)	Tergitol 4	"	"
(15)	Tergitol 08	Synthetic primary alcohol sulfate	"
(16)	Glycerol-mono-oleate	.....	"
(17)	Triethanolamine	.....	"
(18)	Calagon	Sodium hexametaphosphate	Calgon, Incorporated
(19)	Calagon (Unrefined)	"	"
(20)	Triton-B #1	Sulfonated aromatic ether alcohol	Rohm and Haas Company
(21)	Nacconol LA	Sodium alkyl aryl sulfonate	National Aniline & Chemical Co.
(22)	Nacconol NRSF	"	"
(23)	50% Sulfonated castor oil	Sulfonated vegetable oil	Jordan and Company
(24)	75% Sulfonated castor oil	"	"
(25)	50% Sulfonated olive oil	"	"
(26)	75% Sulfonated olive oil	"	"
(27)	Sulfonated red oil	Sulfonated hydrocarbon	"
(28)	Sulphatate	"	Glyco Products Company
(29)	Wetanol	Modified sulfated fatty ester acid	"
(30)	Viriform A		"
(31)	Trimethylol aminomethane	Fatty acid salts of	Commercial Solvents Corp.
(32)	2-amino-2-methyl-1-propanol	"	"

No.	Product	Type	Manufacturer
(33)	2-amino-2-ethyl-1, 3 propanediol	"	"
(34)	Drene	Fatty alcohol sulfate	Procter and Gamble
(35)	Dreft	"	"
(36)	Terposol 8	Compound with sulfonated oil base	Hercules Powder Company
(37)	Daintex	Miscible terpene alcohols and hydrocrabons	"
(38)	Yarmor Pine Oil 302	Terpene alcohol, terpene hydrocarbon, pine oil fraction	"
(39)	Pine Oil 1	High sulfate castor oil and pine oil	"
(40)	Pine Oil 9	Lower sulphated castor oil and higher percentage pine oil	"
(41)	Penetrex	Pine-oil cresylic acid compound in sulfonated oil base	"
(42)	Invadine N	Sodium alkyl phenylene sulfonate	Ciba Company, Inc.
(43)	Invadine C	"	"
(44)	Invadine NC	"	"
(45)	Ultravon W	Sulfonate of complex stearyl alkyl compound	"
(46)	Santomerse D	Alkylated aryl sulfonate	Monsanto Chemical Company
(47)	Santomerse 3	Salt of substituted aromatic sulfonic acid	"
(48)	Monoamylamine	.....	Sharples Solvents Corp.
(49)	Monobutylamine	.....	"
(50)	Mixed amyl chloride	.....	"
(51)	Dichloro pentane	.....	"
(52)	Diamyl sulfide	.....	"
(53)	Triethanolamine stearate	.....	Revson and Company
(54)	Triethanolamine oleate	.....	"
(55)	Sodium ricinoleate (pract.)	.....	Eastman Kodak Company
(56)	Sodium undecylenate	.....	**********
(57)	Sodium laurate	.....	**********
(58)	Sodium palmitate	.....	**********
(59)	Sodium chloride (C. P.)	.....	Merck and Company
(60)	Glycerin (U. S. P.)	.....	"

..... : Chemical names are self-explanatory.

********** : These soaps were made in the Research Laboratory of Sharp and Dohme, Inc., from the chemically pure acids.

ADDENDA "B".

List of Surface Tension Depressants employed for *in vivo* testing:

1) Aerosol OT
2) Tergitol 08
3) Ultrawet
4) Duponol WA
5) Duponol ME
6) Calgon (Unrefined)
7) Nacconol LA
8) Nacconol NRSF
9) Santomerse D
10) Santomerse 3
11) Sulfonated Castor Oil (50%)
12) 2-amino-2-ethyl-1, 3 propanediol

CONTROLS:

1) Sodium ricinoleate
2) Sodium undecylenate
3) Sodium laurate
4) Sodium palmitate
5) Sodium chloride
6) Glycerin

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THE EFFECT OF AEROSOL OT AND HYDROGEN-ION CONCENTRATION ON THE BACTERICIDAL EFFICIENCY OF ANTISEPTICS

By Louis Gershenfeld † and David Perlstein †

Introduction

Disinfection:

OUR modern conception of disinfection began almost half a century ago with Henle's (1) (1889) study of the bactericidal action of Creolin upon the organisms causing Asiastic Cholera and Typhoid Fever. Henle considered disinfection as a chemical reaction between the bacterial cell and the disinfectant.

In 1891, Abbott, (2) in his studies with the Staphylococcus aureus was impressed by the gradual death rate of this organism. Since all of the organisms were suspended in the same concentration of the disinfectant and equally exposed, he concluded that those "cells of equal resistance would die at the same time," whereas those of a greater or less resistance would take a longer or shorter period of time respectively for their destruction.

Which of these viewpoints, the chemical of Henle or the biological of Abbott, is valid, still lacks a conclusive answer, although a considerable amount of data and many theories have been advanced to support each conception.

Lee and Gilbert (3) (1918), while attempting to apply the mass law to the process of disinfection, found that the differences in individual resistance attributed to bacteria by Bellei, Eijkman, Hewlett, Reichel and others, would "assume a very unusual distribution in that the great majority of the population would be the most susceptible." This view was supported by Boycott (4) (1920) and by Fantus and Rumry (5) (1920), who did not find that the time required for disinfection was in proportion to the number of bacteria present. Later, Traube and Somogyi (6) (1921), reported that a strictly chemical interpretation of disinfection was not justified. They claimed that this phenomenon should be attributed to physical effects, as surface forces, adsorption, and electrical potential. The trend today appears to be in the direction of a physical interpretation of disinfection as is evidenced by the great amount of work conducted in recent years with surface tension depressants in

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combination with antiseptics (7). These investigations are evidence to the fact that a decrease in surface tension with the resultant increase of the rate of diffusion results in an increase in the destruction of bacteria by chemical disinfectants.

Surface Tension:

Surface tension is based upon the mutual tendency of the molecules of every liquid to attract each other. This phenomenon plays an important part in the germicidal ability of some disinfectants, whereas others are practically independent of this surface force. Thus, if a substance which appears to lower surface tension is dissolved in a liquid, the molecules of such reducing substance will be forced out of the liquid by the stronger mutual attraction of the molecules of the liquid. On this account, the molecules of the surface tension reducing substance will tend to accumulate at the interface between the liquid and other objects, such as bacteria or inanimate objects as the walls of the container, etc. If the substance in the concentration of the film or membrane surrounding the bacterial cell is toxic, the organism may be killed even though the concentration of such substance in the liquid itself is much lower than the lethal dose. Thus, theoretically, substances which are only moderately toxic but are good surface tension depressants may be found to be effective disinfectants. Hexylresorcinol (S. T. 37) (8) is a disinfectant displaying this characteristic.

A few workers, in personal communications, have stated that instead of obtaining an increase in the efficiency of antiseptics by the addition of a surface tension depressant, the opposite result or variable results were noted (9). In fact in this issue of this journal, Gershenfeld and Witlin (10) in a report on an investigation of surface tension reducents obtained variable findings more than a year ago (although reported at this time), the outgrowth of which resulted in the starting of this present research. It is in part the purpose of this investigation to demonstrate the reason for the disparity in such results obtained by different workers.

Hydrogen-ion Concentration:

Since the presentation of the first studies in bactericidal action, it has been recognized that acidity is an important factor. The classical work of Pasteur (1879) contains suggestions as to the effects of acid on bacterial growth. It has been shown that for any

given species of bacterium there is a definite and frequently narrow pH range which allows vigorous growth (11). This optimum pH for most bacteria usually lies slightly on the alkaline side (about 7.2-7.6). Nevertheless, growth of the most commonly-observed organisms is possible over a pH range of from 5.0 to 8.0.

The study of the bactericidal power of hydrogen-ions began with the work of Paul and Kronig (12) in 1896. They studied the action of mercuric chloride in the presence of salts of metals, acids, and bases, and were the first to indicate that bactericidal action in acid media is proportional to the hydrogen-ion concentration or the degree of electrolytic dissociation. Winslow and Lochridge (13) in 1906 corroborated the findings of Paul and Kronig. They calculated the pH of the solutions they studied by means of conductivity data. However, all of these early workers were handicapped by the lack of more exacting methods for the direct determination of hydrogen-ion concentration. With the subsequent introduction of apparatus and development of techniques, the results of the above workers were checked and corroborated by accurate scientific determinations.

This relationship between pH value and antiseptic action was subsequently studied by many investigators. Joachimoglu (16) found mercuric chloride to be most effective over a pH range of 5.0 to 6.6 and practically ineffective from pH 7.8 to 10.1. Many acids like salicylic, benzoic, aromatic acids, and phenols were investigated by Vermast (17), Waterman and Kuiper (18), and Kuroda (19). Each of these workers concluded that the free acids are much better than the sodium salts at pH values from 3.4 to 8.4. Tetrault (20) studied the antiseptic action of Mallophene (*b*-phenylazo-a-diamino-pyridine hydrochloride) and found a minimum effectiveness at a pH value of 6.6 and an increasing effectiveness at lower pH values. Later, Carroll, Lewis, and Kappel (21) adjusted the pH of mandelic acid solutions in urine and found that highly acid media gave good results. Stearn and Stearn (22) demonstrated an increase in the bactericidal efficiency of acidic dyes with an increase in pH.

Recently (1938), Goshorn, Degering, and Tetrault, (23) working on the bacteriostatic and bactericidal action of benzoic acid, postulated a "hydrogen-ion effect" which increases the efficiency of a compound up to a maximum. These workers later (1939) (24), (1940) (25) confirmed their previous findings in a study involving a diversified group of commercial antiseptics and again found a

"definite increase in efficiency with an increase in hydrogen-ion concentration."

Detergents:

A more complete historical sketch of soaps and synthetic detergents (so-called wetting agents) is available in other papers published and to be published by Gershenfeld and coworkers. For this study, it is sufficient to point out that the bactericidal action of synthetic detergents was demonstrated by Domagk (26) in 1935 when he showed that the detergent and antiseptic Zephiran (alkyl dimethyl benzyl ammonium chloride) possesses excellent germicidal properties. Similar reports then followed in rapid succession. Katz and Lipsitz (27) demonstrated the inhibition of the growth of Mycobacterium smegmatis by a detergent derived from naphthalene sulfonic acid. In 1940 Shelton et al, (28) and Blubaugh, Botts, and Gerwe (29) reported that another detergent, cetyl pyridinium chloride, also possesses germicidal activity.

Purpose

After a thorough perusal of the status of the foregoing, this investigation was started to study the influence of hydrogen-ion concentration upon some surface tension depressants and the effectiveness of certain antiseptics at various pH values by themselves and in combination with a surface tension depressant at these same hydrogen-ion concentrations.

Experimental

Subsequent to a study of the characterististics of a number of the 300 (30) surface tension depressants now commercially available, it was decided to use Aerosol OT, a product of the American Cyanamid and Chemical Corporation, which the available literature indicated to have the following advantages. It is: 1—practically a chemically pure product (99.5-100 per cent. Aerosol OT); 2—practically non-hygroscopic; 3—practically neutral in reaction; 4—sterile in a one per cent. aqueous solution; 5—relatively stable to both acid and alkali; 6—probably non-toxic to human beings when used either internally or externally; 7—reported to be bactericidal.

Preliminary tests were conducted to determine the bactericidal and bacteriostatic properties of this detergent. The details of this work and the significance of hydrogen-ion concentration in the evaluation of these properties were reported in a recent publication by

Gershenfeld and Perlstein (31). The F. D. A. (32) technique for testing antiseptics was used throughout this investigation, and the Kolmer (33) technique was used to test for bacteriostasis.

All tests were conducted at a temperature of 37 degrees C. This is a more exacting temperature because heat tends to dissociate surface tension complexes and to decrease the extent of absorption. Surface tension depressants therefore have less tendency to increase their activity at higher temperatures than other types of compounds due to the fact that they are absorbed to a greater extent at low temperatures.

Every time a test was conducted, the resistance of the Staphylococcus aureus culture was tested against Phenol at 37 degrees C., and if the F. D. A. requirements were not met, the results of that particular test were discarded and the test repeated.

TABLE I
AEROSOL OT

pH of diluent (distilled water)	6.4		6.8	
Time in minutes	5	10	5	10
Dilution				
1:100	—	—	+	+
1:400	—	—	+	+
1:800	—	—	+	+
1:1,000	—	—	+	+
1:2,000	+	—	+	+
1:3,000	+	+	+	+
1:4,000	+	+	+	+
1:5,000	+	+	+	+
1:6,000	+	+	+	+
1:7,000	+	+	+	+
1:8,000	+	+	+	+
1:9,000	+	+	+	+
1:10,000	+	+	+	+
Control:				
Distilled H_2O	+	+	+	+

— indicates no growth (i. e.—a killing effect by the dilution indicated)
+ indicates growth (i. e.—no killing effect by the dilution indicated)

It will be noted (see table 1) that Aerosol OT is a bactericidal agent in aqueous solution killing Staphylococcus aureus in ten but not in five minutes in a concentration of 1:2,000 when using ordinary distilled water at pH 6.4 as the diluent. However, an aqueous solution upon standing at room temperature reveals after a few days a slight decrease in the efficiency of this compound probably due to a slow hydrolysis of the ester Aerosol OT.

Over a period of about one month, the distilled water in our laboratory, collected as is commonly practiced, varied from pH 6.4

to 6.8. The above test was repeated when the distilled water was at the latter extreme (pH 6-8). The findings revealed that even a fresh 1:100 aqueous solution of Aerosol OT at pH 6.8 did not kill Staphylococcus aureus in five minutes.

A fresh aqueous solution of Aerosol OT was then adjusted colorimetrically to various pH values. In each case, the distilled water, the Aerosol OT solution, and the Staphylococcus aureus culture were adjusted to the desired pH before making the dilutions for the actual test. The pH was checked colorimetrically after the completion of the test as an assurance that the desired pH had been attained.

Although, in general, there are two methods for the adjustment and determination of pH, the colorimetric (14) method rather than the potentiometric (15) method was employed. The former was found more convenient and in some instances more accurate for the purpose of this investigation.

TABLE 2

AEROSOL OT

pH of the final mixture	7.0		6.0		5.0		4.0	
Time in minutes	5	10	5	10	5	10	5	10
Dilution								
1:100	+	+	—	—	—	—	—	—
1:500	+	+	—	—	—	—	—	—
1:1,000	+	+	—	—	—	—	—	—
1:2,000	+	+	—	—	—	—	—	—
1:3,000	+	+	—	—	—	—	—	—
1:4,000	+	+	—	—	—	—	—	—
1:5,000	+	+	+	—	—	—	—	—
1:6,000	+	+	+	+	—	—	—	—
1:7,000	+	+	+	+	—	—	—	—
1:8,000	+	+	+	+	—	—	—	—
1:9,000	+	+	+	+	—	—	—	—
1:10,000	+	+	+	+	—	—	—	—
1:15,000	+	+	+	+	—	—	—	—
1:20,000	+	+	+	+	—	—	—	—
1:25,000	+	+	+	+	—	—	—	—
1:30,000	+	+	+	+	—	—	—	—
1:35,000	+	+	+	+	+	—	—	—
1:40,000	+	+	+	+	+	+	—	—
1:45,000	+	+	+	+	+	+	+	—
1:65,000	+	+	+	+	+	+	+	—
1:75,000	+	+	+	+	+	+	+	+
1:100,000	+	+	+	+	+	+	+	+
Control:								
Distilled H_2O	+	+	+	+	+	+	+	+

In table 2, it will be observed that the bactericidal efficiency of Aerosol OT increases with an increase in the hydrogen-ion concen-

tration. An identical experiment was conducted with a fresh one per cent. aqueous solution of Tergitol-4, a product of the Carbide and Carbon Chemical Corporation, with similar findings. However, Tergitol-4 apparently decomposes in aqueous solution after standing at room temperature for approximately 48 hours. It becomes cloudy and then almost milky white as the age of the solution increases. The decomposition of Tergitol-4 was noted when the above experiment was repeated 48 hours later, at which time Tergitol-4 did not kill Staphylococcus aureus even in a one per cent. aqueous concentration. (One per cent. stock solutions of the various detergents were used because they were found most convenient, economical, and in all probability the strongest concentration which would be used in practice.)

It was decided to limit all subsequent experiments to the use of Aerosol OT on account of the above findings. The latter required a relatively long period of time for partial decomposition of its aqueous solutions, and furthermore these solutions revealed greater bactericidal efficiencies at the various pH values than solutions of any of the other reportedly non-toxic surface tension depressants tested (31).

The following concentrations of Aerosol OT used were found to allow a safety range for an assurance that when combined with an antiseptic, the final concentration of Aerosol OT at that pH would not in itself be bactericidal: pH 7.0 (dilution 1:5,000); pH 6.0 (dilution 1:12,000); pH 5.0 (dilution 1:60,000); pH 4.0 (dilution 1:90,000); and pH unadjusted (dilution dependent upon the resultant pH of the mixture).

Six compounds were selected which were considered to be representative of broad groups of antiseptics: Phenol, Mercuric Chloride, Merthiolate Solution (1:1,000), Hexylresorcinol (1:1,000), Zephiran Solution (1:1,000), and Zonite. The bactericidal and bacteriostatic efficiencies of these compounds were first tested at each pH value without the addition of Aerosol OT, and then repeated with the addition of the proper non-bactericidal concentration of Aerosol OT for each pH value. The Aerosol OT and sterile distilled water were added to the respective antiseptics in such a manner that the final mixture of each dilution contained both the required concentration of the antiseptic and of the Aerosol OT.

The pH of the aqueous 1:100 stock solution of Aerosol OT, of the respective antiseptic, of the distilled water used to make the

dilutions, and of the Staphylococcus aureus culture was adjusted to the desired value immediately prior to performing the test, so that the final mixture had a pH as reported in the tables. Adjustments were made with N/1 or N/10 HCl or NaOH V. S. After the completion of the test, the pH of the mixture was checked colorimetrically.

In the subsequent tables "+" indicates growth and "—" indicates no growth.

TABLE 3

PHENOL

pH of the final mixture	7.0		not adjusted (6-7)		6.0		5.0		4.0	
Time in minutes	5	10	5	10	5	10	5	10	5	10
Dilution										
1:60	—	—	—	—	—	—	—	—	—	—
1:65	—	—	—	—	—	—	—	—	—	—
1:70	—	—	—	—	—	—	—	—	—	—
1:75	—	—	—	—	—	—	—	—	—	—
1:80	+	—	+	—	—	—	—	—	—	—
1:85	+	+	+	—	+	—	—	—	—	—
1:90	+	+	+	+	+	+	+	—	—	—
1:95	+	+	+	+	+	+	+	—	—	—
1:100	+	+	+	+	+	+	+	+	+	+
1:110	+	+	+	+	+	+	+	+	+	+
1:120	+	+	+	+	+	+	+	+	+	+
1:150	+	+	+	+	+	+	+	+	+	+
1:200	+	+	+	+	+	+	+	+	+	+
Control:										
Distilled H_2O	+	+	+	+	+	+	+	+	+	+

PHENOL WITH AEROSOL OT

pH of the final mixture	7.0		not adjusted (6-7)		6.0		5.0		4.0	
Conc. of Aerosol	1:5,000		1:7,000		1:12,000		1:60,000		1:90,000	
Time in minutes	5	10	5	10	5	10	5	10	5	10
Dilution										
1:60	—	—	—	—	—	—	—	—	—	—
1:80	—	—	—	—	—	—	—	—	—	—
1:100	—	—	—	—	—	—	—	—	—	—
1:120	—	—	—	—	—	—	—	—	—	—
1:140	—	—	—	—	—	—	—	—	—	—
1:180	+	—	—	—	—	—	—	—	—	—
1:200	+	+	+	+	—	—	—	—	—	—
1:240	+	+	+	+	+	+	—	—	—	—
1:280	+	+	+	+	+	+	—	—	—	—
1:300	+	+	+	+	+	+	+	—	—	—
1:340	+	+	+	+	+	+	+	+	—	—
1:380	+	+	+	+	+	+	+	+	+	—
1:400	+	+	+	+	+	+	+	+	+	+
1:450	+	+	+	+	+	+	+	+	+	+
1:500	+	+	+	+	+	+	+	+	+	+
Controls:										
Distilled H_2O	+	+	+	+	+	+	+	+	+	+
Aerosol OT	+	+	+	+	+	+	+	+	+	+

TABLE 4

MERCURIC CHLORIDE

pH of the final mixture	7.0		not adjusted (6-7)		6.0		5.0		4.0	
Time in minutes	5	10	5	10	5	10	5	10	5	10
Dilution										
1 :10,000	—	—	—	—	—	—	—	—	—	—
1 :15,000	+	—	—	—	—	—	—	—	—	—
1 :17,000	+	+	—	—	—	—	—	—	—	—
1 :19,000	+	+	+	—	—	—	—	—	—	—
1 :20,000	+	+	+	+	—	—	+	—	—	—
1 :25,000	+	+	+	+	+	+	+	—	—	—
1 :30,000	+	+	+	+	+	+	+	+	—	—
1 :35,000	+	+	+	+	+	+	+	+	+	—
1 :40,000	+	+	+	+	+	+	+	+	+	—
1 :45,000	+	+	+	+	+	+	+	+	+	+
1 :50,000	+	+	+	+	+	+	+	+	+	+
1 :75,000	+	+	+	+	+	+	+	+	+	+
1 :100,000	+	+	+	+	+	+	+	+	+	+
Control:										
Distilled H_2O	+	+	+	+	+	+	+	+	+	+

MERCURIC CHLORIDE WITH AEROSOL OT

pH of the final mixture	7.0		not adjusted (6-7)		6.0		5.0		4.0	
Time in minutes	5	10	5	10	5	10	5	10	5	10
Conc. of Aerosol	1 :5,000		1 :7,000		1 :12,000		1 :60,000		1 :90,000	
Dilution										
1 :10,000	—	—	—	—	—	—	—	—	—	—
1 :15,000	+	—	—	—	—	—	—	—	—	—
1 :20,000	+	—	—	—	—	—	—	—	—	—
1 :25,000	+	+	+	—	—	—	—	—	—	—
1 :30,000	+	+	+	+	+	+	—	—	—	—
1 :35,000	+	+	+	+	+	+	+	—	—	—
1 :40,000	+	+	+	+	+	+	+	+	—	—
1 :45,000	+	+	+	+	+	+	+	+	+	—
1 :50,000	+	+	+	+	+	+	+	+	+	—
1 :55,000	+	+	+	+	+	+	+	+	+	+
1 :60,000	+	+	+	+	+	+	+	+	+	+
1 :75,000	+	+	+	+	+	+	+	+	+	+
1 :100,000	+	+	+	+	+	+	+	+	+	+
Controls:										
Distilled H_2O	+	+	+	+	+	+	+	+	+	+
Aerosol OT	+	+	+	+	+	+	+	+	+	+

TABLE 5

MERTHIOLATE

pH of the final mixture	not adjusted (8-9)		7.0		6.0		5.0		4.0	
Time in minutes	5	10	5	10	5	10	5	10	5	10
Dilution										
1:3,000	—	—	—	—	—	—	—	—	—	—
1:5,000	—	—	—	—	—	—	—	—	—	—
1:7,000	+	+	+	—	+	+	—	—	—	—
1:10,000	+	+	+	+	+	+	—	—	—	—
1:15,000	+	+	+	+	+	+	—	—	—	—
1:18,000	+	+	+	+	+	+	+	+	—	—
1:20,000	+	+	+	+	+	+	+	+	—	—
1:25,000	+	+	+	+	+	+	+	+	—	—
1:30,000	+	+	+	+	+	+	+	+	+	—
1:35,000	+	+	+	+	+	+	+	+	+	+
1:40,000	+	+	+	+	+	+	+	+	+	+
1:45,000	+	+	+	+	+	+	+	+	+	+
1:50,000	+	+	+	+	+	+	+	+	+	+
Control:										
Distilled H_2O	+	+	+	+	+	+	+	+	+	+

MERTHIOLATE WITH AEROSOL OT

pH of the final mixture	not adjusted (8-9)		7.0		6.0		5.0		4.0	
Conc. of Aerosol	1:5,000		1:5,000		1:12,000		1:60,000		1:90,000	
Time in minutes	5	10	5	10	5	10	5	10	5	10
Dilution										
1:3,000	—	—	—	—	—	—	—	—	—	—
1:5,000	—	—	—	—	—	—	—	—	—	—
1:7,000	—	—	—	—	—	—	—	—	—	—
1:10,000	—	—	—	—	—	—	—	—	—	—
1:15,000	+	—	—	—	—	—	—	—	—	—
1:20,000	+	—	—	—	—	—	—	—	—	—
1:25,000	+	+	—	—	—	—	—	—	—	—
1:30,000	+	+	—	—	+	—	—	—	—	—
1:35,000	+	+	+	+	+	+	+	—	—	—
1:40,000	+	+	+	+	+	+	+	+	—	—
1:45,000	+	+	+	+	+	+	+	+	—	—
1:50,000	+	+	+	+	+	—	+	+	+	+
Controls:										
Distilled H_2O	+	+	+	+	+	+	+	+	+	+
Aerosol OT	+	+	+	+	+	+	+	+	+	+

TABLE 6

HEXYLRESORCINOL

pH of the final mixture	7.0		6.0		5.0		4.0		not adjusted (3-4)	
Time in minutes	5	10	5	10	5	10	5	10	5	10
Dilution										
1:1,000	—	—	—	—	—	—	—	—	—	—
1:2,000	—	—	—	—	—	—	—	—	—	—
1:3,000	+	—	—	—	—	—	—	—	—	—
1:4,000	+	+	+	+	—	—	—	—	—	—
1:5,000	+	+	+	+	—	—	—	—	—	—
1:8,000	+	+	+	+	+	+	—	—	—	—
1:12,000	+	+	+	+	+	+	—	—	—	—
1:15,000	+	+	+	+	+	+	+	+	—	—
1:18,000	+	+	+	+	+	+	+	+	+	+
1:20,000	+	+	+	+	+	+	+	+	+	+
1:30,000	+	+	+	+	+	+	+	+	+	+
Control:										
Distilled H_2O	+	+	+	+	+	+	+	+	+	+

HEXYLRESORCINOL WITH AEROSOL OT

pH of the final mixture	7.0		6.0		5.0		4.0		not adjusted (3-4)	
Conc. of Aerosol	1:5,000		1:5,000		1:12,000		1:60,000		1:90,000	
Time in minutes	5	10	5	10	5	10	5	10	5	10
Dilution										
1:4,000	—	—	—	—	—	—	—	—	—	—
1:10,000	—	—	—	—	—	—	—	—	—	—
1:20,000	—	—	—	—	—	—	—	—	—	—
1:25,000	+	+	+	—	+	—	—	—	—	—
1:30,000	+	+	+	+	+	—	—	—	—	—
1:35,000	+	+	+	+	+	+	—	—	—	—
1:40,000	+	+	+	+	+	+	+	+	—	—
1:45,000	+	+	+	+	+	+	+	+	+	+
1:50,000	+	+	+	+	+	+	+	+	+	+
Controls:										
Distilled H_2O	+	+	+	+	+	+	+	+	+	+
Aerosol OT	+	+	+	+	+	+	+	+	+	+

TABLE 7

ZONITE

pH of the final mixture	not adjusted (8-9)		7.0		6.0		5.0		4.0	
Time in minutes	5	10	5	10	5	10	5	10	5	10
Dilution										
1:200	—	—	—	—	—	—	—	—	—	—
1:300	—	—	—	—	—	—	—	—	+	—
1:400	—	—	—	—	—	—	—	—	+	—
1:500	—	—	—	—	—	—	—	—	+	+
1:600	—	—	—	—	—	—	+	—	+	+
1:800	—	—	—	—	+	+	+	+	+	+
1:1,000	—	—	—	—	+	+	+	+	+	+
1:2,000	—	—	+	—	+	+	+	+	+	+
1:3,000	+	+	+	+	+	+	+	+	+	+
1:4,000	+	+	+	+	+	+	+	+	+	+
1:5,000	+	+	+	+	+	+	+	+	+	+
Control:										
Distilled H_2O	+	+	+	+	+	+	+	+	+	+

ZONITE WITH AEROSOL OT

pH of the final mixture	not adjusted (8-9)		7.0		6.0		5.0		4.0	
Conc. of Aerosol	1:5,000		1:5,000		1:12,000		1:60,000		1:90,000	
Time in minutes	5	10	5	10	5	10	5	10	5	10
Dilution										
1:100	—	—	—	—	—	—	—	—	—	—
1:400	—	—	—	—	—	—	—	—	—	—
1:500	—	—	—	—	—	—	—	—	+	—
1:600	—	—	—	—	—	—	—	—	+	+
1:800	—	—	—	—	+	—	+	+	+	+
1:1,000	—	—	—	—	+	+	+	+	+	+
1:2,000	—	—	—	—	+	+	+	+	+	+
1:3,000	—	—	+	—	+	+	+	+	+	+
1:4,000	+	+	+	+	+	+	+	+	+	+
1:5,000	+	+	+	+	+	+	+	+	+	+
Controls:										
Distilled H_2O	+	+	+	+	+	+	+	+	+	+
Aerosol OT	+	+	+	+	+	+	+	+	+	+

TABLE 8

ZEPHIRAN

pH of the final mixture	not adjusted (7.0-7.4)		7.0		6.0		5.0		4.0	
Time in minutes	5	10	5	10	5	10	5	10	5	10
Dilution										
1:5,000	—	—	—	—	—	—	+	+	+	+
1:10,000	—	—	—	—	—	—	+	+	+	+
1:20,000	—	—	—	—	+	—	+	+	+	+
1:30,000	—	—	—	—	+	+	+	+	+	+
1:40,000	—	—	—	—	+	+	+	+	+	+
1:50,000	—	—	—	—	+	+	+	+	+	+
1:60,000	—	—	—	—	+	+	+	+	+	+
1:70,000	—	—	—	—	+	+	+	+	+	+
1:80,000	+	+	+	+	+	+	+	+	+	+
1:90,000	+	+	+	+	+	+	+	+	+	+
1:100,000	+	+	+	+	+	+	+	+	+	+
Control:										
Distilled H_2O	+	+	+	+	+	+	+	+	+	+

ZEPHIRAN WITH AEROSOL OT

The above experiment was repeated with the addition of Aerosol OT in concentrations as reported in the previous tables. All tubes showed growth.

Dilutions of Phenol in concentrations stronger than 1:100 (see table 3) were prepared from a standardized 1:20 stock solution of Phenol (Reagent, Merck). Weaker dilutions were made from the 1:100 solution.

Dilutions of Mercuric Chloride in concentrations stronger than 1:10,000 (see table 4) were prepared from a standardized 1:1,000 aqueous stock solution of Mercuric Chloride (Reagent, Merck). Weaker dilutions were made from the 1:10,000 solution.

Dilutions of Merthiolate in concentrations stronger than 1:10,000 (see table 5) were prepared from a 1:1,000 aqueous Solution of Merthiolate as marketed. Weaker dilutions were made from the 1:10,000 solution.

Dilutions of Hexylresorcinol in concentrations stronger than 1:10,000 (see table 6) were prepared from a 1:1,000 aqueous Solution of Hexylresorcinol as marketed. Weaker dilutions were made from the 1:10,000 solution.

Dilutions of Zonite in concentrations stronger than 1:1,000 (see table 7) were prepared from a 1:100 aqueous stock solution of Zonite. Weaker dilutions were made from the 1:1,000 solution.

Dilutions of Zephiran in concentrations stronger than 1:10,000 were prepared from a 1:1,000 aqueous Solution of Zephiran as marketed. Weaker dilutions were made from the 1:10,000 solution.

Many other dilutions of each antiseptic were prepared and tested. These were concentrations either stronger or weaker than noted in the different tables. Though these dilutions were not included in the recorded tables, attention is being directed to the fact that all concentrations stronger than the weakest dilution revealing a *killing effect* were tested and also found to possess identical bactericidal characteristics as the latter. In like manner, many dilutions weaker than those revealing *no killing effect* (i. e. did not kill) were tested and the results were found to be identical (i. e. did not kill).

Surface Tension:

Surface tension measurements were made of the mixtures of the various antiseptics studied in combination with Aerosol OT at the various pH values, using the DuNouy tensiometer. The findings were not found to be significantly or consistently different. The surface tension measurements made of the highest killing dilution in ten but not in five minutes of the antiseptic solutions containing Aerosol OT and organisms at the various pH values, revealed nothing significant.

Discussion

Zonite, as shown in table 7, did not exert a significant increase in bactericidal efficiency when Aerosol OT was added to it at the various pH values. On the basis of the theory of the mode of action of surface tension depressants, the above finding may be expected because the action of Zonite depends upon the free chlorine liberated and not necessarily upon a solution whose penetrability into the bacterial cell is aided by a reduction in interfacial tension. There undoubtedly is a chemical reaction between Aerosol OT and Zonite. The alkalinity of Zonite hydrolyzes the ester (Aerosol) forming Octyl Alcohol. Although the latter in itself reduces surface tension, the actual total reduction in interfacial tension is not as great as that of the unhydrolized Aerosol OT. The Zonite is destroyed and the available chlorine is lost.

Zephiran, as shown in table 8, was the only antiseptic tested that actually showed a marked decrease in bactericidal efficiency

when Aerosol OT was added to it at the various pH values. This incompatibility is explained in a communication from the Alba Pharmaceutical Company, the manufacturers of Zephiran, as follows: "Aerosol OT is the sodium salt of an acid while Zephiran is the chloride of a base; consequently the Zephiran salt of Aerosol OT would be formed, which would be insoluble and would not show any bactericidal action. This salt is, of course, an ammonium salt substituted by the radicals present in our Zephiran."

In the tables it will be noted that upon the addition of a non-bactericidal concentration of a detergent as Aerosol OT to certain antiseptics, and the mixtures adjusted to an acid pH, an increase in the bactericidal efficiency of the mixtures will result with an increase in the hydrogen-ion concentration. It is difficult to give an exact explanation of the mechanism by which this action takes place. The data which has been accumulated is so varied and yet in each instance based upon definite findings, that a consideration of this nature becomes highly speculative. The pH effect may be due to one, or several factors, as noted by different workers. (34) The formation of undissociated molecules may be accelerated and these may be more effective than ions because of their greater ability to enter the bacterial cell. Osterhout (35) (1935) demonstrated that only undissociated molecules penetrate the cell membrane. The explanation may be on the basis of greater adsorption. Phelps and Peters (36) showed that the adsorption of fatty acids on charcoal depends on the concentration of undissociated acid. Stearn and Stearn (37) contend that hydrogen-ion concentration directly affects the bacteria, altering either membrane or protoplasm in such a manner as to render the organism more susceptible to the action of the detergent. That hydrogen-ion concentration may influence the formation of micelles (colloidal ions), was demonstrated by Hartley (38) (1936). It is known that detergents may change from molecular to micellar solution. Other theories and data as to the factors responsible for the pH effect have been advanced.

The surface tension measurements leaves an inference in accordance with the general contention that bactericidal action is aided by interfacial tension and not surface tension. Unfortunately, interfacial tension between the chemical mixtures and the bacterial cell is not directly measurable, so that a conclusion cannot be reached on this important point.

Summary

The bactericidal and bacteriostatic properties of Aerosol OT were studied in aqueous solutions where the hydrogen-ion concentrations were adjusted to known and varied values.

Dilutions of six antiseptics, namely: Phenol, Mercuric Chloride, Solution Merthiolate (1:1,000), Hexylresorcinol (1:1,000), Zonite, and Solution Zephiran (1:1,000), were tested both by themselves and in combination with non-bactericidal concentrations of Aerosol OT at various pH values.

Surface tension measurements were made of the antiseptics studied, the aqueous solutions (fresh and old) of Aerosol OT, and combinations of the antiseptics with Aerosol OT and organisms at the different pH values and of the various killing dilutions.

All tests were checked and rechecked with freshly prepared and old aqueous solutions on several occasions, so that the findings reported are the results of many tests.

Conclusion

Of the six antiseptics studied in combination with a non-bactericidal concentration of Aerosol OT at the various pH values, the following exert, *in vitro,* a marked increase in bactericidal efficiency with an increase in the hydrogen-ion concentration of the mixture: Phenol, Bichloride of Mercury, Solution Merthiolate (1:1,000), and Hexylresorcinol (1:1,000). Zonite and Zephiran Solution (1:1,000), due to their chemical incompatibility with Aerosol OT do not exert a significant increase and in some instances especially in the case of Zephiran even show a decrease in bactericidal efficiency for reasons given (chemical incompatibility).

The following dilutions kill Staphylococcus aureus at 37° C. within five minutes at the pH noted:

Phenol

Without Aerosol OT	With Aerosol OT (conc. see table 3)
unadjusted (pH 6-7), 1:75	unadjusted (pH 6-7), 1:180
adjusted to pH 4.0, 1:95	adjusted to pH 4.0, 1:340

Mercuric Chloride

Without Aerosol OT	With Aerosol OT (conc. see table 4)
unadjusted (pH 6-7), 1:17,000	unadjusted (pH 6-7), 1:20,000
adjusted to pH 4.0, 1:30,000	adjusted to pH 4.0, 1:40,000

Merthiolate

Without Aerosol OT	With Aerosol OT (conc. see table 5)
unadjusted (pH 8-9), 1:5,000	unadjusted (pH 8-9), 1:10,000
adjusted to pH 4.0, 1:25,000	adjusted to pH 4.0, 1:45,000

Hexylresorcinol

Without Aerosol OT	With Aerosol OT (conc. see table 6)
unadjusted (pH 3-4), 1:15,000	unadjusted (pH 3-4), 1:40,000
adjusted to pH 7.0, 1:2,000	adjusted to pH 7.0, 1:20,000

Zonite

Without Aerosol OT	With Aerosol OT (conc. see table 7)
unadjusted (pH 8-9), 1:2,000	unadjusted (pH 8-9), 1:3,000
adjusted to pH 4.0, 1:200	adjusted to pH 4.0, 1:400

Zephiran

Without Aerosol OT	With Aerosol OT (conc. see table 8)
unadjusted (pH 7-7.4), 1:70,000	unadjusted (pH 7-7.4), all dilutions showed growth
adjusted to pH 4.0, all dilutions showed growth	adjusted to pH 4.0, all dilutions showed growth

Weaker dilutions in most instances are effective at the same pH as in the foregoing if the ten-minute period is noted.

Stronger concentrations of the antiseptic (containing the same amount of a non-bactericidal percentage of Aerosol OT) are required to exert a bactericidal effect, within the five and ten-minute periods, when the hydrogen-ion concentration is decreased approaching neutrality.

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SOLID EXTRACTS

Prepared from Current Literature by the Editor and his Staff

It doesn't seem fair to impose upon our readers a repetition of the old saying that an apple a day keeps the doctor away and that an onion will keep everybody away, but if one were to substitute garlic for the apple, the saying would more likely be true, especially if the patient were suffering from high blood pressure. To determine the efficacy of this substance a New York physician experimented on a series of standardized cats (human vascular hypertension fluctuates too much from day to day to allow for good statistical results), and he found that intraperitoneal injections of garlic concentrate promptly reduce the blood pressure in every instance, even more so than allyl sulfide, garlic's active principle. Further, it was found that these injections inhibited the normal epinephrine response, indicating a quite thorough job.

AJP

Tung oil, that ancient and honorable substance deriving its name from the Chinese word for heart because of the shape of the plant's leaves, and first taken from its Oriental habitat in 1516, has risen to a respectable but costly place in the industrial world. Consequently, it has been replaced in traffic pavement paints by a newcomer, namely soybean oil. The latter is cheaper, more pliable, and meets every requirement for quick drying and durability.

AJP

The relatively new Food, Drug and Cosmetic Act is producing some marvelous results, and is creating a general atmosphere of mutual confidence. There is yet much to be done, however. Witness the current newspaper ads such as "Could Victoria Reign 63 Years With Stomach Ulcer Pains?" and "Could Adam Eat the Apple With Stomach Ulcer Pains?" These edifying insertions in the daily press continue with admonitions not to ignore sufferings, but try a 25¢ box of a certain brand of tablets for relief of ulcer and stomach pains, indigestion, gas pains, heartburn, burning sensation, bloat and other conditions caused by excess acid. They must help or your money refunded. Yes, there is much still to be done!

In this respect, the authorities should be complimented for their quick action on almost 3000 medicinal substances submitted for their official verdict. Only about 300 are still under consideration, and only three have been turned down completely. Compliments should go, too, to the manufacturers who are trying so hard to comply with all the new rules and regulations and who are, as attested to by the small rejection list, doing a good job.

AJP

This next extract has come to us from the laboratory of practical experience, and is intended only for our readers of the weaker sex who have taken advantage of one of science's masterpieces and are wearing Nylon stockings. If they will also take the trouble to wear light weight fabric gloves while taking these stockings off or putting them on, they will avoid nasty fingernail and abrased skin snags and add even more mileage to their already phenomenal endurance records.

AJP

The world's first oil well was drilled in 1859 in Venango County, Pa., by E. L. Drake, a railroad conductor who saw opportunities for bigger and better things than punching tickets and calling stations. Chemists and pharmacists the world over have seen to it that these opportunities materialized far beyond Mr. Drake's fondest expectations.

AJP

For more than two years, two Chicago doctors have been making clinical tests of the possibility of combining immunization against tetanus and diphtheria. In observations on 240 children so immunized in that period, positive results have been noted. Basic immunization may be obtained by two injections of 1 cc. of the combined tetanus and diphtheria toxoids or three injections of 0.5 cc. each. Intervals of three months or more between injections bring optimum response. Even before the present critical observation period, large numbers of humans were doubly immunized and no cases among them of the diseases against which they were fortified have yet been reported. Thus, while the period of immunization is still indeterminate, it is probably considerable.

Increased acidosis produced by sulfanilamide, and not by sulfapyridine and sulfathiazole, is explained by the fact that the sulfonamide end of the former's molecule makes carbonic anhydrase inactive. Carbonic anhydrase is a red blood cell enzyme which separates carbonates into carbon dioxide and water, with the gas escaping through the lungs. A lack of carbon dioxide, in turn, may well be a factor in the development and progress of arthritis. Investigations are being conducted along these lines in a western Pennsylvania hospital.

OUR CONTRIBUTORS THIS MONTH

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